

# Airborne Stability of Tailless Bacterial Viruses S-13 and MS-2

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The effect of relative humidity (RH) on the airborne stability of two small bacterial viruses, S-13 and MS-2, was studied. Poorest recovery of S-13 was obtained at 50% RH. Humidification prior to aerosol sampling significantly increased the recovery of S-13 at RH deleterious to the airborne virus. A commercial preparation of MS-2 suspended in a buffered saline solution showed a rapid loss of viability at RH above 30%, whereas a laboratory preparation containing 1.3% tryptone showed high recoveries at all RH studied. Dilution of the commercial MS-2 into tryptone broth conferred stability on the airborne virus. Humidification prior to sampling significantly reduced the viable recovery from aerosols of commercial MS-2, whereas the laboratory preparation was unaffected.

Most research on the airborne stability of bacterial viruses has utilized the T-series of bacterial viruses (7, 10, 13, 19) which possess a unique taillike structure permitting attachment to bacteria. Factors that damage this structure during or after aerosolization eliminate the particle as an infective unit. These same factors, however, may not pertain to airborne viruses without this complex tail structure.

To retain the simplicity of the bacteria-virus system and to obtain information which might apply to animal viruses, two unusual bacterial viruses, S-13 and MS-2, were selected for studies on airborne stability. These were chosen because of physical characteristics that closely resemble many animal viruses: (i) both are 230 to 300 nm with apparent icosahedral symmetry (5, 17, 18, 22); (ii) both contain single-stranded nucleic acids, S-13 [deoxyribonucleic acid (DNA)], MS-2 [ribonucleic acid (RNA); references 5, 21]; and (iii) both lack a complex tail structure. This report concerns the effect of different relative humidity (RH) values on the airborne survival of S-13, MS-2, and a commercially prepared stock of MS-2.

## MATERIALS AND METHODS

**Media.** Growth media, overlay, and agar plates were prepared by the method of Tessman (20). All strains of bacteria and viruses were prepared in the same media, which consisted of NaCl, 7 g; tryptone, 13 g; water, 1 liter (tryptone broth).

**Growth and assay of viruses.** Bacteriophage S-13 and its host bacterium, *Escherichia coli* C, were obtained from Irwin Tessman of Purdue University.

Lysates were prepared by the method of Tessman (20) and were maintained at 4 C.

Bacteriophage MS-2 and its host bacterium, *E. coli* C 3000, were obtained from Robert Sinsheimer of the California Institute of Technology. Viral lysates were prepared by adding virus to host cells in the exponential growth phase. After 4 to 5 hr, lysozyme-ethylenediaminetetraacetic acid [(EDTA) 0.05 mg/ml,  $5 \times 10^{-3}$  M] was added to liberate the virus. Cellular debris was removed by centrifugation. Lysates were stored at 4 C. Titers of the lysates ranged between  $10^{12}$  and  $10^{13}$  plaque-forming units (PFU) per ml.

A commercially prepared stock of MS-2 virus was obtained from Miles Laboratory, Elkhart, Ind. The virus was suspended in 0.1 M NaCl, 0.05 M tris(hydroxymethyl)aminomethane (pH 7.6), and 0.001 M EDTA (salt solution). The initial titer was  $8 \times 10^{14}$  PFU/ml. For aerosol work, the commercial stock of MS-2 was diluted in salt solution or tryptone broth to give a final concentration of  $10^9$  PFU/ml.

Viral assays were by the plaque method of Adams (1). Because the plaques of MS-2 were small, a minimal amount of agar overlay (2 ml) was used. To this was added 0.2 ml of a diluted 12-hr culture of host cells. Plates were incubated at 37 C immediately after plating. Those plates remaining at room temperature for 0.5 hr prior to incubation at 37 C showed reduced plaque numbers. Because plaque number also showed variation with the age of the agar plates, a consistent age of agar plates was used for all experiments.

**Aerosol equipment.** Aerosols were held in an NBL 500-liter rotating toroid drum (9) at a holding temperature of 21 C. At least three experiments were made for each RH selected. A modified Wells reflux atomizer was operated for 10 min at a pressure of 15 psi (13). Antifoam (Dow Corning Antifoam B) was added (to give a final 1% concentration) to viral suspensions containing tryptone prior to aerosolization to prevent

excess foaming. After a 10-min drum-fill time, the drum was allowed to equilibrate for an additional 5 min prior to sampling.

A sample was obtained immediately as the aerosol left the atomizer en route to the drum. The age of the aerosol at this sampling point was approximately 5 sec. Recovery from this sample was considered 100% unless otherwise specified. It was observed that the aerosol concentration at the 5-sec sampling point was greater than that obtained from within the drum at 15 min (the first drum sampling time). This difference was noted by using the light scatter method of Dimmick, Hatch, and Ng (6), and recovery values were adjusted to correct for this difference in concentration.

Aerosol samples of 1 min each were collected at 15-min intervals over a period of 2 hr using either the AGI-30 impinger alone or in combination with a humidifier bulb (13), with the following modification. The 2,000-ml flask had open stems on both sides with the aerosol entering and leaving from opposite sides. The AGI-30 impinger fluid consisted of 20.5 ml of tryptone broth plus 1% of antifoam. Virus content was expressed as plaque forming units per liter of air. Curves derived from drum experiments begin with the first drum sample because the exact shape of the curve prior to this time can not be determined in these experiments.

## RESULTS

Previous studies (23) indicated that airborne S-13 had a mid-range (40 to 60%) RH sensitivity within the Dynamic Aerosol Transport Apparatus (12). This was also shown to be true for S-13 aerosols aged in an NBL toroid drum (Fig. 1). At 50% RH, S-13 aerosols had lost 99% of their infectivity by the end of 15 min, when first sampled from the drum. Recovery from an aerosol held at 20% RH was approximately 10-fold greater than at 50% RH but still accounted for less than 10% of the original aerosol infectivity ( $3 \times 10^6$  to  $5 \times 10^6$  PFU/liter of air). At 80% RH, airborne S-13 was very stable. Use of the AGI-30-humidifier bulb combination (AG-30-HB) for sampling S-13 aerosols resulted in significantly increased recoveries of viable virus at both 20 and 50% RH.

One possible reason for the increased recovery of viable viruses observed with the AG-30-HB combination was that prehumidification increased the efficiency of the AGI-30 impinger. One type of inefficiency would be a change in collection efficiency over a range of aerosol concentrations. To test this, the concentration of S-13 suspensions was varied over a 6-log range prior to atomization. Using only the AGI-30, 5-sec aerosols were collected at 80% RH (Fig. 2). The slope of the line is 1, indicating that there was no

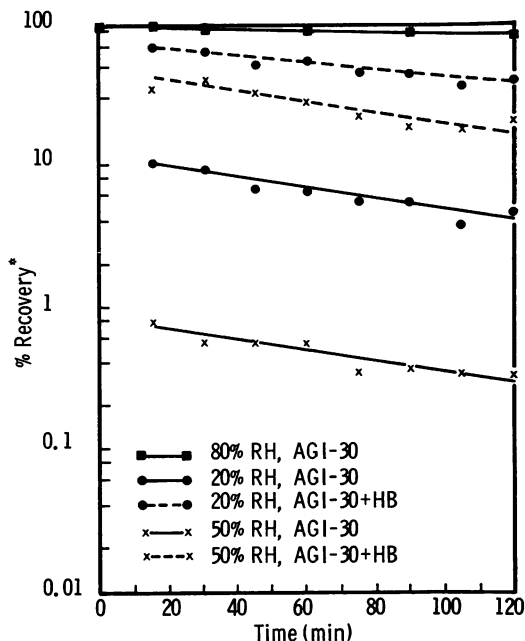


FIG. 1. Recovery of airborne S-13 suspended in tryptone broth plus 1% Antifoam B at different relative humidities; \* 100% recovery is  $3 \times 10^6$  to  $5 \times 10^6$  PFU/liter of air.

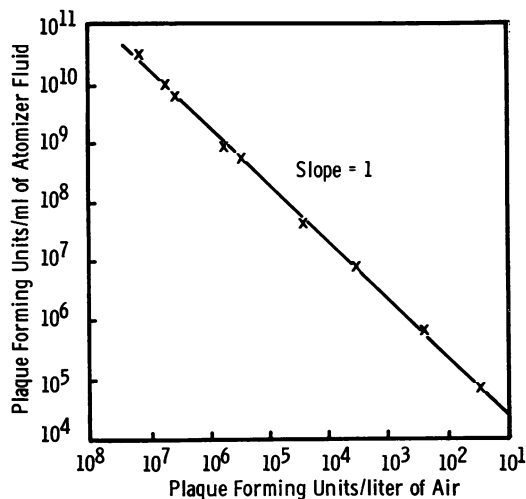


FIG. 2. Recovery of 5-sec S-13 aerosols at 80% RH using variation in aerosol concentrations with the AGI-30 impinger.

change in collection efficiency for the AGI-30 over the range of aerosol concentrations tested.

Initial aerosol experiments with MS-2 in salt solution resulted in unusually low recoveries. Accordingly, MS-2 aerosols sampled at 5 sec were studied over a range of RH from 20 to

80% (Fig. 3). At above 30% RH, there was a marked decrease in the recovery of viable airborne MS-2 particles. Drum studies (2 hr; Fig. 4) at 20, 50, and 80% RH revealed that the airborne inactivation pattern observed at 5 sec was maintained; i.e., low RH (20%) gave the greatest recoveries, whereas mid-range gave the least. In contrast, 5-sec and 2-hr aerosols derived from MS-2 replicated, suspended, and aerosolized in tryptone broth resulted in high recoveries at all RH values tested.

The prehumidification technique for sampling S-13 aerosols enhanced recoveries at those RH values deleterious to airborne S-13. This was not true for MS-2 aerosols (Table 1, condition A). Instead, for MS-2 aerosols derived from MS-2 in salt solution there was a decrease in recovery by this technique. MS-2 aerosols prepared in tryptone broth did not respond to prehumidification (Table 1, condition B).

Experiments were undertaken to determine what effect either or both tryptone and Antifoam B would have on the airborne stability of MS-2 virus in salt solution. As can be seen in Fig. 5, Antifoam B (final concentration of 1%) added to the MS-2 in salt solution conferred an ini-

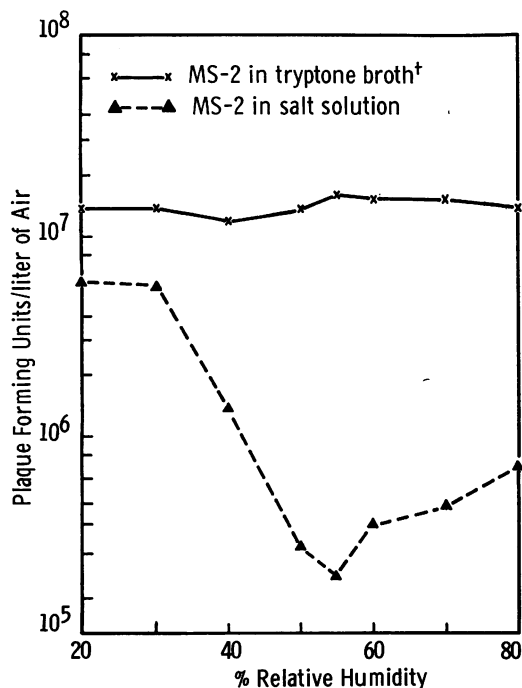


FIG. 3. Recovery of MS-2 virus from 5-sec aerosols at different relative humidity values. †MS-2 replicated and atomized in tryptone broth.

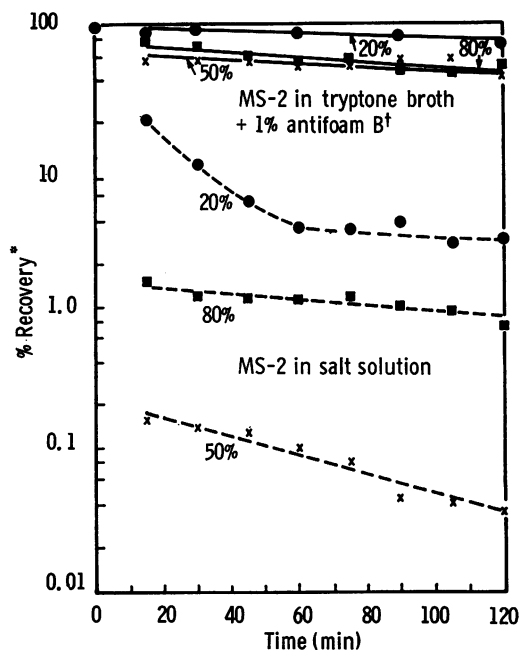


FIG. 4. Recovery of MS-2 virus aerosolized in two different spray suspensions; \*100% recovery for MS-2 in salt solution is  $10^6$  PFU/liter of air, and 100% recovery for MS-2 in tryptone broth is  $4 \times 10^6$  to  $6 \times 10^6$  PFU/liter of air. †MS-2 replicated and atomized in tryptone broth.

TABLE 1. Comparison of two sampling methods<sup>a</sup>

Sampling method	Aerosol age (min)		
	60	90	120
Condition A <sup>b</sup>			
AGI-30.....	$4.1 \times 10^4$	$4.5 \times 10^4$	$3.9 \times 10^4$
AGI-30 plus humidifier bulb...	$3.8 \times 10^2$	$1.0 \times 10^2$	$1.2 \times 10^2$
Condition B <sup>c</sup>			
AGI-30.....	$1.5 \times 10^5$	$1.2 \times 10^5$	$5.8 \times 10^4$
AGI-30 plus humidifier bulb...	$1.1 \times 10^5$	$1.1 \times 10^5$	$5.1 \times 10^4$

<sup>a</sup> Values are expressed as plaque-forming units per liter of air.

<sup>b</sup> Recovery of airborne MS-2 virus aerosolized in salt solution at 20% relative humidity.

<sup>c</sup> Recovery of airborne MS-2 aerosolized in tryptone broth at 20% relative humidity.

tial, although transitory, stability. After 2 hr, the recovery approached that for aerosols generated without antifoam. With the dilution of MS-2 virus in salt solution into tryptone broth, it was necessary to add antifoam because of excess foaming during atomization. The effect of tryptone broth and 1% antifoam is similar to

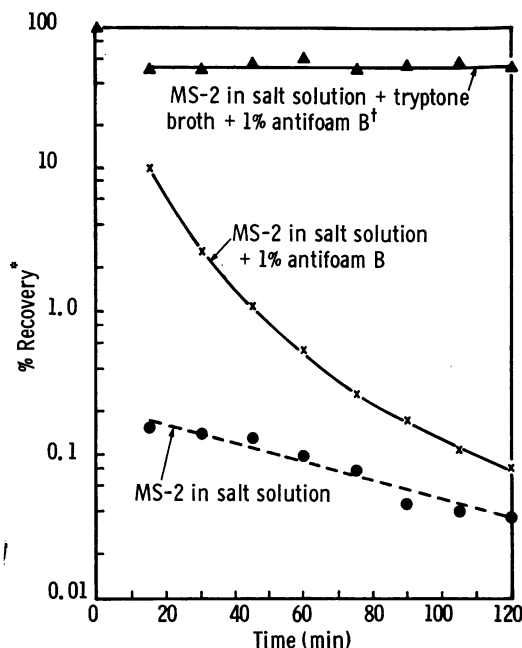


FIG. 5. Recovery of MS-2 virus in various spray suspensions at 50% RH; \*100% recovery is  $10^6$  PFU/liter of air. †MS-2 in salt solution diluted into tryptone broth plus 1% Antifoam B.

that observed with aerosols derived from virus replicated and prepared for atomization in tryptone broth supplemented with antifoam (Fig. 5).

### DISCUSSION

The use of S-13 and MS-2 in studying the mechanism(s) of inactivation of airborne viruses appears justified because of the similarity in airborne behavior which exists between these two bacterial viruses and certain animal viruses. For instance, the encephalomyocarditis-group viruses [Columbia-SK and mengovirus-37A (2, 3)], vesicular stomatitis (24), Rous sarcoma (25), measles (15), and poliovirus (11, 14) exhibit patterns of mid-range RH sensitivity as observed with S-13 and MS-2.

The extreme loss of viable MS-2 aerosolized in salt solutions and stability after aerosolization in tryptone broth solution were not surprising. Harper (11) found that poliovirus sprayed in NaCl and KCl solutions gave airborne recoveries similar to crude tissue culture lysates at low RH, but evinced reduced recoveries at higher RH (50 and 80%). More recently, Benbough (4), aerosolizing Semliki Forest virus, reported that recoveries were greatly increased at intermediate and high RH when salt was removed from the

virus suspension. As reported herein, the addition of a protein hydrolysate stabilizes airborne MS-2. This only serves to confirm the reports of Harper (11), Webb et al. (25), and Benbough (4) which indicated that almost any compound that is added to a spray fluid may influence the resultant aerosol stability of the virus in question.

Because of their single-stranded nucleic acid structure and the ease of preparing high titers, S-13 and MS-2 appear to be ideal candidates for studies in which aerosols of intact virions could be compared with aerosols of their infectious nucleic acids. Data from such studies might indicate further information on the mechanism of airborne viral inactivation which, to date, is still conflicting. De Jong and Winkler (16) recently reported that the infectivity of the RNA of poliovirus decays in the same manner as the intact virus. Infectious RNA in this case was extracted from impinger samples, allowing a comparison to be made between surviving intact virions and recoverable infectious RNA. The authors ascribed the inactivation of airborne poliovirus to the "denaturation" of the RNA. This is difficult to imagine since the infectivity of infectious RNA appears to be independent of its secondary structure.

Akers and Hatch (3) produced aerosols of infectious mengovirus RNA and found that it was stable in the RH regions in which the intact virus was unstable. This work showed that the RNA molecule itself was stable in the airborne state. It seems, therefore, that the inactivation of the RNA, if it occurs, is a secondary event, with the primary event being a conformational change in the protein capsid. The increased recovery of S-13 by humidification prior to sampling supports this idea. S-13 contains a cyclic single strand of DNA whose biological activity is chiefly dependent upon its primary structure (8). Repair of the primary structure of a nucleic acid molecule by a change in relative humidity does not appear feasible. It is, however, highly likely that dehydration and rehydration cause changes in the conformation of the proteins in the virion.

### ACKNOWLEDGMENTS

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